(2-16-95)

MRID No. 429024-03

DATA EVALUATION RECORD

- Oxine Copper. CHEMICAL: Shaughnessey No. 024002.
- TEST MATERIAL: 1) Quinolate 98; oxine copper or copper 8-2. quinolinolate; Batch No. 52390; 100% active ingredient; a green powder. 2) $^{14}\text{C-oxine copper}$; Lot No. 029F9233; specific activity of 92.9 $\mu\text{Ci/mg}$; 98.25% active ingredient. 3) 14C-oxine copper; Lot No. 041H9267; specific activity of 63.78 μ Ci/mg; 98% active ingredient.
- 3. STUDY TYPE: 72-4. Freshwater Fish Early Life-Stage Toxicity Test. Species Tested: Rainbow Trout (Oncorhynchus mykiss).
- CITATION: Lintott, D.R. and G.S. Ward. 1993. Oxine Copper (Copper 8-quinolinolate): Toxicity to Embryos and Larvae of the Rainbow Trout, Oncorhynchus mykiss, Under Flow-Through Test Conditions. Project No. J9006014e. Performed by Toxikon Environmental Sciences, Jupiter, FL. Submitted by LA QUINOLEINE et ses dérivés, S.A., Paris, France. EPA MRID No. 429024-03.

5. REVIEWED BY:

Mark A. Mossler, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.

APPROVED BY: 6.

> Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.

Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA

Signature: / May Justen 1/16/95
Date: 12/13/9

Signature:

Date:

CONCLUSIONS: This study is not scientifically sound and does not fulfill the guideline requirements. The percent RSD of weight in one replicate of the solvent control was >40%. Under the conditions of the test, the MATC, based upon mean measured concentrations, was >1.69 and <3.51 µg ai/l of oxine copper due to significant reductions in length and wet weight at the highest treatment level. The geometric mean MATC was 2.44 μg ai/1.

- 8. RECOMMENDATIONS: N/A.
- 9. BACKGROUND:
- 10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
- 11. MATERIALS AND METHODS:
 - A. <u>Test Animals</u>: Rainbow trout (Oncorhynchus mykiss) eggs and milt were obtained from a commercial supplier in Red Bluff, CA. Upon receipt, the gametes were gently mixed in plastic bowls. Cold dilution water was then added to activate the sperm. An "egg wash" (tris buffer, glycerin, and salt) was added followed by several cold water rinses. Four hours were allowed for egg hardening.
 - B. Test System: The test system was a modified proportional vacuum-siphon diluter with a 50% dilution factor. Two replicates per treatment level and control were used. The test chambers were 24-1 glass tanks (40 x 29.5 x 20 cm) containing 15 l of test solution at a depth of 13 cm. The diluter cycled an average of 2.8 times per hour providing approximately 4.5 volume additions per day.

Embryo incubation cups were glass petri dishes with Nitex screen (350 μ m) collars. Two cups were used per replicate. The flow of test solution into the test chamber was directed into the incubation cups.

The chambers were randomly positioned in a water bath surrounded by black curtains under fluorescent and incandescent light. Developing embryos and newly hatched fry were maintained in darkness until swim-up had been attained. At swim-up, an 8-hour light photoperiod was initiated for the first week and this was increased the second week to 16 hours of light per day. Light intensity gradually increased to 391-498 lux. Fifteen-minute periods of low light were provided to simulate dawn and dusk.

The dilution water was a blend of treated town of Jupiter (FL) water and well water which was aerated. The town water had been aerated, filtered (5 $\mu m)$, passed through activated carbon, and treated by reverse

osmosis. The well water was passed through activated carbon. During the test, the water had a specific conductivity of 601-1,667 μ mhos/cm, and a hardness and alkalinity of 64-200 and 22-123 mg/l as CaCO₃, respectively.

A stock solution containing 17.5% radiolabeled material and 82.5% unlabeled material was prepared in acidified dimethylformamide (DMF). The concentration of oxine copper in the stock solution was 1 mg active ingredient (ai)/ml. The stock solution was pumped into the diluter mixing chamber providing a high nominal test concentration of 4 μ g ai/l. The mixing chamber solution was proportionally diluted to provide the lower-concentration treatment solutions.

- C. <u>Dosage</u>: Ninety-five-day, flow-through test. Nominal test concentrations selected based on results of preliminary testing were 0.25, 0.50, 1.0, 2,0, and 4.0 μ g ai/l. A dilution water control and solvent control were also prepared. The solvent control and exposure solutions contained a DMF concentration of 4 μ l/l.
- Design: Within 5 hours of fertilization, embryos were impartially distributed to the incubation cups, by twos, until each cup contained 20 eggs for a total of 80 embryos per treatment level or control. Two additional incubation cups containing 50 embryos were added to each replicate control tank to determine fertilization success. Survival of embryos was recorded daily until hatching was complete. After hatching, the fish were released into the test tanks.

Post swim-up larvae were fed live brine shrimp nauplii two to three times daily. Flake food was added to the diet on day 65. Feeding of brine shrimp was discontinued on day 79. Feeding was discontinued the day prior to test termination.

Behavior, appearance, and survival of larvae were noted daily. At test termination, standard length and wet weight of each individual were measured.

Dissolved oxygen concentration (DO) and pH were measured in each test solution at test initiation and weekly thereafter. Hardness, alkalinity, and conductivity of the dilution water were measured weekly. Temperature was monitored hourly in the dilution water control using a data logging device.

The daily temperature range of the water bath was determined using a minimum/maximum thermometer.

Water samples were collected at test initiation and weekly thereafter from each replicate. The concentration of oxine copper was determined using liquid scintillation counting.

- E. <u>Statistics</u>: Hatch and survival data were arcsine square root transformed prior to analysis. Statistical differences between control and treatment groups were determined using analysis of variance (ANOVA) coupled with Dunnett's test. Statistical calculations were performed at the 0.05 probability level. The most sensitive parameter was used to determine the maximum acceptable toxicant concentration (MATC).
- 12. REPORTED RESULTS: Mean measured concentrations were 0.173, 0.434, 0.792, 1.69, and 3.51 μ g ai/l (Tables 1 and 2, attached). These values ranged from 69 to 88% of nominal concentrations. No undissolved test material was observed in the treatment chambers during the test.

Based on the extra sets of eggs, fertilization success in dilution water was approximately 99%. Hatching began on day 30 and was completed by day 49. The day selected for hatch completion was day 35, when ≥95% of the control embryos had hatched. Hatching success averaged 91 and 94% in the dilution water and solvent controls, respectively (Table 3, attached), and was not affected by exposure to oxine copper (86-100% hatch in the treatment solutions). A few abnormalities were noted (Siamese twins and backbone deformities), but there was no apparent concentration relationship.

Juvenile survival in the dilution water control and solvent control averaged 99 and 89%, respectively (Table 4, attached). Survival in the treatment groups ranged between 59 and 97%, and was not significantly different from the control survival.

The summarized growth data were presented in Table 5 (attached). Solvent control fish growth was significantly lower than dilution water control fish growth. Mean wet weight and length of fish at the 3.51 μ g ai/l level were significantly lower than those of the solvent control.

The test temperature during the 95-day exposure ranged from 9.1 to 12.8°C. The DO remained ≥ 7.1 mg/l ($\geq 7.8\%$ of

saturation). The pH was 7.5-8.5 in all test containers during the test.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
Based upon a reduction in growth (length and wet weight),
the MATC was >1.69 and <3.51 μg ai/l. The geometric mean
MATC was 2.44 μg ai/l. The no-observed-effect concentration
(NOEC) was 1.69 μg ai/l.

A Good Laboratory Practice (GLP) statement was included in the report, indicating that the study was conducted in accordance with EPA GLP regulations set forth in 40 CFR Part 160. A Quality Assurance statement was also presented.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. <u>Test Procedure</u>: This study generally followed the recommended guidelines. Deviations from the SEP were as follows:

The percent RSD of wet weight for replicate B of the solvent control was 45.3%; the percent RSD of weight must not be greater than 40%, according to the SEP.

The method by which the solvent concentration was made equal in all exposure solutions was not described.

Hardness, alkalinity, and conductivity should have been measured in at least one exposure solution.

The SEP recommends four replicates per concentration, only two replicates were used in this test.

Embryos were "impartially" distributed; the SEP requires random selection.

B. <u>Statistical Analysis</u>: Percent hatch and fry survival data were analyzed using one-way ANOVA and Williams' test. The results were the same as those of the authors' (see attached printouts 1 and 2). However, upon visual examination of the data, there appeared to be a substantial reduction in survival at the highest treatment level.

Fish weight and length were analyzed using two-way ANOVA and Bonferroni's test. Compared to the solvent control, exposure to 3.51 μ g ai/l of oxine copper significantly reduced fish length (printouts 3 and 4, attached) and fish wet weight (printouts 5 and 6,

- attached). Based on mean measured concentrations, the NOEC and lowest-observed-effect concentration (LOEC) were 1.69 and 3.51 μg ai/l, respectively.
- C. <u>Discussion/Results</u>: This study is not scientifically sound and does not fulfill the guideline requirements. The percent RSD of weight in one replicate of the solvent control was >40%. Based upon mean measured concentrations, the MATC was >1.69 and <3.51 μ g ai/l of oxine copper due to significant reductions in length and wet weight at the highest treatment level. The geometric mean MATC was 2.44 μ g ai/l.

D. Adequacy of the Study:

- (1) Classification: Invalid.
- (2) Rationale: The percent RSD of weight in one replicate of the solvent control was >40%.
- (3) Repairability: No.
- 15. COMPLETION OF ONE-LINER: Yes, 11-22-93.

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rainbow trout hatching success

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	sol cont	2	0.940	1.339	1 274
2	0.173	2	0.965	1.387	1.374 1.374
3	0.434	2	0.955	1.366	1.374
4	0.792	2	0.905	1.287	1.374
5 6	1.69	. 2	1.000	1.492	1.374
	3.51	2	0.865	1.195	1.195

rainbow trout hatching success

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
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0.434	1.374	0.329		2.06	k= 1, v= k= 2, v=
0.792	1.374	0.329		2.10	k= 3, v=
1.69	1.374	0.329		2.12	k = 4, $v =$
3.51	1.195	1.345		2.13	k= 5, v=

s = 0.107

Note: df used for table values are approximate when v > 20.



rainbow trout survival

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N 	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
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rainbow trout survival

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

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1	1	1.0	37.0	WEIGHT 0.66
2	1	1.0	37.0	0.70
3	1	1.0	37.2	0.63
4	1	1.0	37.0	0.59
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26	1	1.0	38.2	0.76
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41	1	2.0	30.0	0.38
42	1	2.0	33.0	0.45
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382	6	1.0	38.0	0.75
383	6	1.0	33.0	0.54
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390	6	2.0	22.0	
391	6	2.0	27.0	0.10
392	6	2.0		0.16
393	6	2.0	30.5	0.27
394	6		28.0	0.28
395	6	2.0	31.0	0.21
396		2.0	26.0	0.13
397	6	2.0	37.0	0.55
	6	2.0	33.0	0.31
398	6	2.0	27.0	0.22
		•		